Microbial Metabolism

Energy – Yielding (Catabolic)

&

Energy – Requiring (Anabolic)

Biochemical Processes

Overview

- Living organisms are, chemical machines – their structure and functions can be traced directly or indirectly to chemical reactions.
- The term *metabolism* denotes all the chemical activities performed by an organism.
- These activities are of two general types:
  - Those involved in liberating energy and
  - Those involved in utilizing energy
- Energy is the ability to do work, and a living cell must perform many different types of work, such as making enzymes, synthesizing a cell wall and cytoplasmic membrane, and repairing damage.
- To do this work, a cell needs a vast amount of energy.
- The source of this energy for some organisms is the nutrient molecules (chemicals) that are taken in by the cell.

Overview

- For other organisms, the source of energy is light; when exposed to light, they convert the energy of light into chemical energy used for metabolism.
- It is important to realize that many of these microbial metabolic mechanisms are also used by higher organisms (including humans) to obtain energy.

Energy requirements of microbial cells

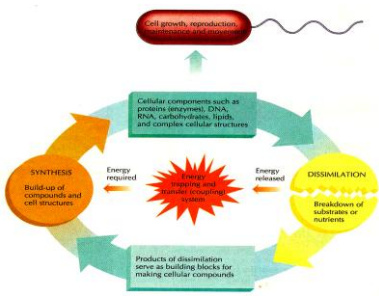
- A living cell requires energy to perform many different kinds of work, including:
  - Construction of the structural parts of the cell such as cell wall, membrane, or external appendages
  - Synthesis of enzymes, nucleic polysaccharides, phospholipids, and other chemical components of the cell
  - Repair of damage and maintenance of the cell in good condition
  - Growth and multiplication
  - Accumulation of nutrients and excretion of waste products
  - Motility
- Most microorganisms obtain energy by *dissimilation,* the breakdown of nutrients or chemical substances
- During dissimilation energy is released from the nutrient molecules and is stored temporarily in an *energy-trapping system* until needed.

Energy requirements of microbial cells

- The energy trapping system also serves as an energy-transfer system that supplies energy when it is needed for synthesis of cell constituents.
- Dissimilation of nutrients molecules also provides the building blocks from which cell constituents can be synthesized.
- Although processes of dissimilation and synthesis are opposite to one another, they are interrelated and proceed concurrently in a microbial cell

Relationships between the processes of dissimilation and synthesis in microbial cells

Relationships between the processes of dissimilation and synthesis in microbial cells. An energy trapping and transfer (coupling) system carries usable energy between the two processes.



Major energy-yielding sources for microorganisms

- *Chemotrophic* microorganisms obtain energy by dissimilating nutrients, or chemical substrates
- *Chemoheterotrophic* microorganisms are chemotrophs that dissimilate *organic* compounds to obtain energy. For example:

Streptococcus lactis (Chemoheterotrophs) + Glucose (Organic substrate) ⟶ Energy + Lactic acid (End product)

Major energy-yielding sources for microorganisms

- *Chemoautotrophic* microorganisms dissimilate *inorganic* compounds to obtain energy. For example:

Nitrosomonas europaea (Chemoautotroph) + ammonia (inorganic substrate) ⟶ Energy + Nitrite (End product)

Major energy-yielding sources for microorganisms

- Some microorganisms that use light as their energy source are called *phototrophs.*
- Phototrophic microorganisms contain special pigments that absorb the light and trap its energy. For example:

Anabaena cylindrica (phototroph) + light ⟶

Absorption of light by cell pigment ⟶ Energy

Chemical Energy and Energy Transfer

- Energy can exist in various forms, *chemical energy* is used universally by living organisms
- *Radiant energy* (the energy of light) can be used by some microorganisms, but these microorganisms must convert the light energy into *chemical energy* in order to have the energy in a form useful for cell functions.
- *Heat energy* (the energy associated with the random motions of molecules or atoms) is a form of energy that cannot be used by living organisms.
- However, a certain amount of heat is necessary in order for chemical reactions even when catalyzed by enzymes, to proceed at rates fast enough to sustain life.

Chemical Energy and Energy Transfer

- For instance, the rate of most enzymes-catalyzed reactions increases by a factor of about 2 for each 10 ^{0}C increases in temperature, up to the temperature at which the particular enzyme begins to deteriorate.

Energy Transfer Between Exergonic and Endergonic Chemical Reactions

- Chemical reactions that liberates energy are called exergonic reactions, whereas chemical reactions that take up energy are called endergonic reactions.
- In a living organism, the exergonic reactions provide the energy to fuel the endergonic reactions.
- In order to link these reactions, organisms have developed a process called energy coupling:

Exergonic reaction liberates energy

↓

A portion of the energy is trapped into an *energy-transfer (coupling) compound*

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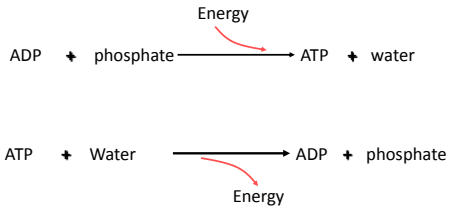
The energy-transfer compound then donates the trapped energy to an endergonic reaction

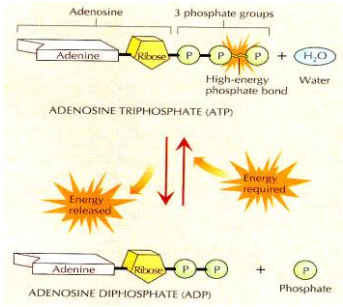
Energy Transfer Between Exergonic and Endergonic Chemical Reactions

- Several kinds of high energy transfer compounds occur in cells, but one is by far the most important: ***adenosine triphosphate (ATP).***



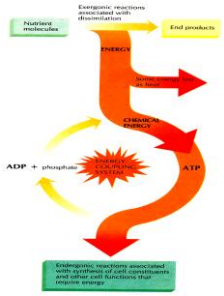
Energy Transfer Between Exergonic and Endergonic Chemical Reactions

ATP is composed of the purine base adenine, the sugar ribose, and three phosphate groups. The third phosphate is linked to the molecule by a high-energy phosphate bond. Breakdown of ATP to ADP releases chemical energy, whereas synthesis of ATP from ADP requires energy.



Energy Transfer Between Exergonic and Endergonic Chemical Reactions

The flow of chemical energy from dissimilation of nutrient molecules to ATP, and then from ATP to the energy-requiring (endergonic) reactions of a microbial cell. Some energy is always lost in the form of heat.



Generation of ATP by Microorganisms

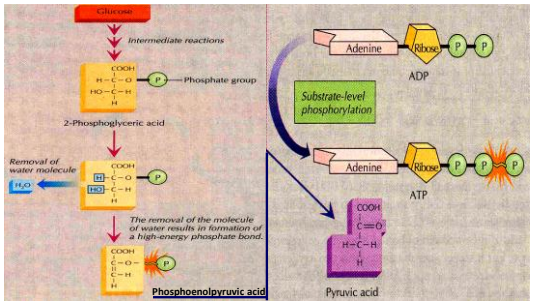
- ***Phosphorylation*** is the addition of a phosphate group to a compound
- ATP is formed by phosphorylation of ADP, with energy for the addition provided by an exergonic reaction.
- There are three general ways in which this phosphorylation of ADP can occur:

1. ***Substrate level phosphorylation*** , a process in which the phosphate group of a chemical compound is removed directly and added to ADP
   - The rearrangement of atoms within chemical compound may result in a new compound that contains a high-energy phosphate bond
   - The phosphate group involved in this bond can then be transferred directly to ADP, forming ATP

Substrate Level Phosphorylation

An example of substrate-level phosphorylation. Many microbial cells can break down glucose to 2-phosphoglyceric acid, and, when an enzyme subsequently removes a molecule of water from 2-phosphoglyceric acid, a molecule of phosphoenolpyruvic acid is formed. The phosphoenolpyruvic acid contains a high energy phosphate bond, and the energy of this bond can be used to transfer the phosphate group directly to ADP to make ATP.

Substrate Level Phosphorylation



Generation of ATP by Microorganisms

2. ***Oxidative phosphorylation,*** a process by which the energy liberated by the chemical oxidations of nutrient chemical compounds is used for the synthesis of ATP from ADP
   - All oxidation reactions liberate energy, and many organisms have developed ways to use the energy from chemical oxidations for ATP synthesis.
   - The sequence of events:

Energy is liberated by an integrated series of sequential chemical oxidation reactions called an *electron-transport system*

↓

The energy is stored temporarily in the form of a *protonmotive force.*

↓

The protonmotive force powers the synthesis of ATP from ADP

Oxidation Reactions

- Oxidation is the loss of one or more electrons from an atom or a molecule, with the electrons being transferred immediately to a recipient atom or molecule.
- In biology, many oxidations involve the loss of a hydrogen atom from a molecule; since a hydrogen atom contains an electron in addition to its proton, a molecule that loses a hydrogen atom has automatically lost an electron.

H (Hydrogen atom (reduced form of atom)) $\longrightarrow$ H^+ (Hydrogen ion (reduced form of atom)) + e^- (Electron (Transferred immediately to a recipient atom or molecule))

Oxidation Reactions

$CH_2(COOH)CH_2(COOH)$ (Succinic acid (reduced form of compound)) $\longrightarrow$ $CH(COOH)=CH(COOH)$ (Fumaric acid (oxidized form of compound)) + $2H$ (Hydrogen atoms (each with an electron))

Fe^{2+} (Ferrous ion reduced from an atom) $\longrightarrow$ Fe^{3+} (Ferric ion (Oxidized from of atom)) + e^- (Electron)

- The opposite of oxidation is reduction, or the gain of electrons (or hydrogen atoms)

Oxidation Reactions

- It is clear that the reverse of any oxidation is a reduction and the reverse of any reduction is an oxidation. In each reaction, a pair of substances is involved – one is the oxidized form, the other the reduced form (e.g., Fe^{3+} and Fe^{2+}, H^+ and H, fumaric acid and succinic acid)
- Each pair of such substances is called an ***oxidation – reduction (O/R) system.***

Electron Transport Systems

- Cells that use the energy of oxidation reactions for ATP synthesis do not rely on a single oxidation reaction that liberates a large burst of energy.
- Instead, a cell uses an integrated series of sequential oxidation reactions called an electron – transport system, which liberates the energy in several smaller more efficient manner.

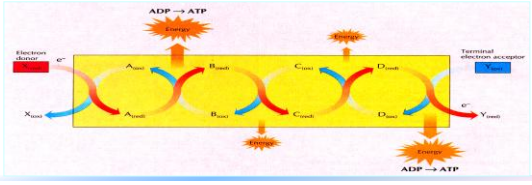
Electron donor → e^- → $(O/R)_1$ → e^- → $(O/R)_2$ → e^- → $(O/R)_3$ → e^- → Terminal electron acceptor

↓

Energy liberated for ATP synthesis

Electron Transport Systems

An electron donor (X_{red}), which may be any variety of the reduced compounds, initially supplies electrons to the electron – transport system and becomes oxidized (X_{ox}). The electrons are released along a series of intermediary O/R systems ($A_{(OX)}/A_{(red)}$, $B_{(OX)}/B_{(red)}$, $C_{(OX)}/C_{(red)}$, $D_{(OX)}/D_{(red)}$, each having greater oxidizing power than the one that precedes it. The electrons eventually reach the terminal electron acceptor, $Y_{(OX)}$, which is an oxidized compound such as oxygen (O_2), potassium nitrate (KNO_3), or potassium sulfate (K_2SO_4). This compound takes up the electrons and becomes reduced (Y_{red}). Energy is released at each oxidation step along the electron transport system, and at some steps the amount of energy released is great enough to allow the synthesis of ATP from ADP.



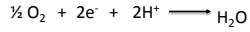
Electron Transport Systems

- The system begins with an *electron donor,* a reduced compound which provides the electrons.
- For instance, some microorganisms use lactic acid as an electron donor:

- The electrons from the electron donor are removed by an initial O/R system.
- This O/R system is in turn oxidized by the next O/R system, and so forth.
- Finally the electrons are taken up by a *terminal electron acceptor,* an oxidized compound obtained from the cell's environment.

Electron Transport Systems

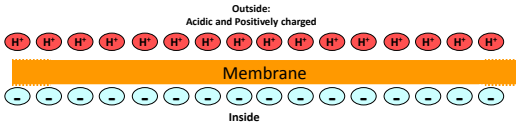
- For instance, aerobic organisms use oxygen as the terminal electron acceptor
- After accepting the electrons from the last O/R system, the oxygen becomes reduced to water:



- Anaerobic organisms that have an electron-transport system do not use oxygen as a terminal electron acceptor; instead, they use a chemical such as nitrate, sulfate, or fumaric acid.

The Protonmotive Force

- In 1978, the biochemist Peter Mitchell received a noble Prize for discovering the way by which energy liberated by an electron-transport system is used for the synthesis of ATP.
- He showed that the energy is used *to pump protons* (hydrogen ions, or H^+) across the membrane where the electron transport is located.
- After the protons are pumped across membrane they can not easily return, because the membrane is not permeable to protons.
- Therefore, the continued operation of an electron-transport system results in accumulation of protons on the one side of the membrane (outside the bacterial cell) and a deficit of protons on the opposite side (inside the bacterial cell).
- The result is that one side of the membrane becomes much more positevely charged and acidic than the other side:

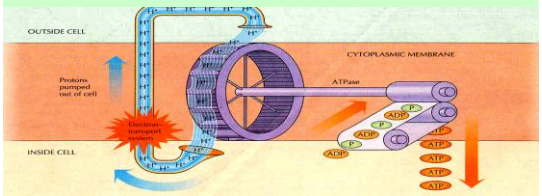


The Protonmotive Force

- This unequal distribution of protons and electric charges across the membrane represents an important form of potential energy called the ***protonmotive force***, which is used to synthesize ATP.
- Certain specific channels do exists in the cytoplasmic membranes that allows passage of protons back to other side of the membrane.
- The proton flow through these channels is harnessed by the cell to do the work of phosphorylating ADP to ATP.
- These channels occur within the molecules of an enzyme called ***adenosine triphosphatase (ATPase),*** which spans the membrane.
- The proton flow forces this enzyme to phosphorylate ADP, thereby forming ATP.

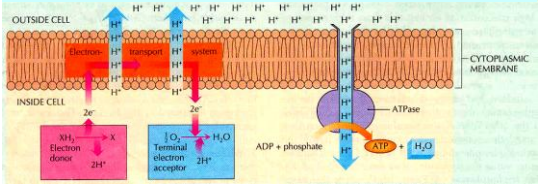
The Protonmotive Force

Schematic drawing illustrating the concept of the protonmotive force by means of a mechanical model. Energy liberated by the oxidation reactions of an electron-transport system is used to pump protons to the outside of the bacterial cytoplasmic membrane. The protons reenter the cell via an enzyme called ATPase, which in turn catalyzes the synthesis of ATP. No mechanical devices like those depicted here are actually present in the cell membranes.



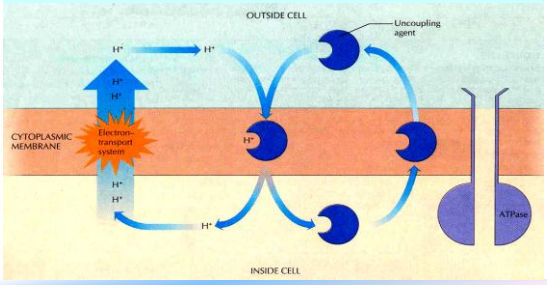
The Protonmotive Force

Schematic representation of an electron-transport system in a bacterial cytoplasmic membrane. Electrons from an electron donor pass along an electron-transport system and eventually reach a terminal electron acceptor (in this case O_2 ,which becomes reduced to water). The energy liberated by the electron transport system is used to pump protons (hydrogen ions, H^+) across the membrane to the outside of the cell, generating a protonmotive force. The protons can return to the inside only by passing through a channel in the enzyme ATPase, which causes the enzyme to synthesize ATP to ADP.



The Protonmotive Force

Uncoupling agents carry protons (hydrogen ions) freely across the cytoplasmic membrane. This prevents proton accumulation outside the bacterial cell and eliminates the protonmotive force that powers ATP synthesis.



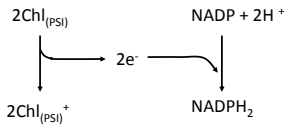
Generation of ATP by Microorganisms

3. ***Photophosphorylation,*** is a process in which the energy of light is used for the synthesis of ATP from ADP:
   - ***Photophosphorylation*** is the overall process in which light is used as a source of energy for ATP synthesis.
   - The general way in which photophosphorylation occurs is as follows:
     1. Light is used to generate a protonmotive force
     2. The protonmotive force then powers ATP synthesis
   - The most important example of photophosphorylation is the type carried out by cynobacteria, algae, and green plants.
   - Cynobacteria, algae, and green plants are also able to use carbon dioxide (CO_2) as their sole source of carbon; that is, they are ***autotrophic organisms.***
   - They reduce the CO_2 to carbohydrate $(CH_2O)_x$ by a process called ***CO_2 fixation.***
   - The $NADPH_2$ is used as an electron donor for reduction of CO_2.
   - The generation of ATP and $NADPH_2$ depends on the activity of two different kinds of chlorophyll-containing reaction centers, called *photosystem I (PS I) and photosystem II (PS II),* which are located in thylakoid membranes.
   - The two photosystems work together in tandem.

Generation of ATP by Microorganisms

- **Three main steps are involved:**

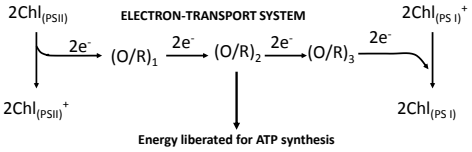
1. When light is absorbed by the chlorophyll molecules in PS I, the energy of light raises the molecules to an excited state, causing an electron to be ejected from each. These electrons are used to reduce NADP to $NADPH_2$:



This leaves the chlorophyll of PS I temporarily deficient in electrons, giving it a positive charge.

Generation of ATP by Microorganisms

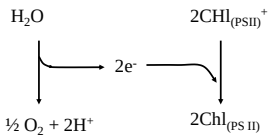
2. Similarly, light is absorbed by the chlorophyll of PS II and causes electrons to be ejected by this photosystem. These electrons pass along an electron-transport system and reach the electron-deficient Chl^+ of PS I:



The result is same as with oxidative phosphorylation – a protonmotive force is generated across the membrane and is used to power ATP synthesis

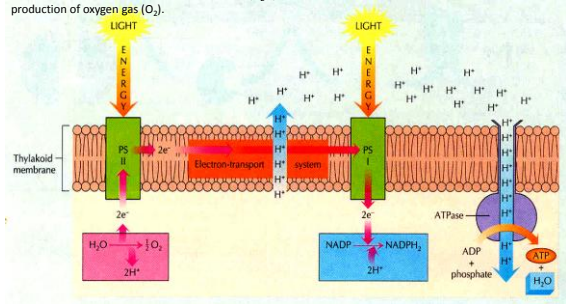
Generation of ATP by Microorganisms

3. At this point, $Chl_{(PSII)}^+$ is still deficient in electrons. However, $Chl_{(PSII)}$ +is a very strong oxidizing agent – so strong that it can regain electron by removing then from molecules of water. This oxidation of water results in the formation of gaseous oxygen:



Generation of ATP by Microorganisms

Schematic diagram showing how light energy is used by cynobacteria for the production of ATP and $NADPH_2$. In the presence of light, electrons are ejected from photosystem I (PSI) and photosystem II (PSII), leaving both photosystem electron – deficient. The electrons ejected from PS I are used to reduce NADP to $NADPH_2$, while those ejected from PS I pass along an electron – transport system and reach PS I. The electron – transport system generates a protonmotive force that causes ATPase to synthesize ATP. The electron – deficient PS II obtains electrons from water (H_2O) molecules, and this oxidation of water results in the production of oxygen gas (O_2).



Pathways for Dissimilation of Nutrients

- Chemotrophic organisms use chemical compounds as an energy source.
- First step is break down of nutrients into compounds that can be used for ATP generation.
- Accomplished by a series of consecutive enzyme-catalyzed chemical reactions called a ***dissimilatory pathway.***
- Serve:
  1. To liberate energy from nutrients, and
  2. To supply many of the building blocks from which a cell can construct its proteins, lipids polysaccharides, and nucleic acids.

Dissimilation of Complex Nutrients

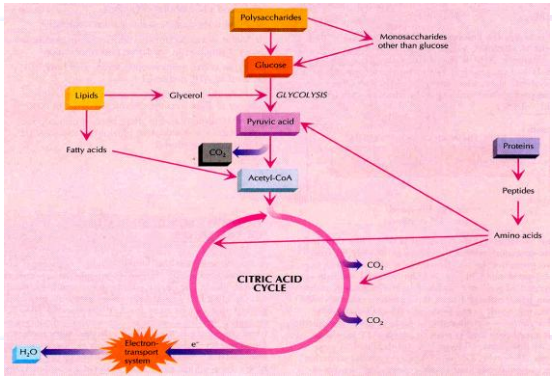
Proteins to Amino acids

Fats to Glycerol and Fatty Acids

Polysaccharides to Monosaccharides

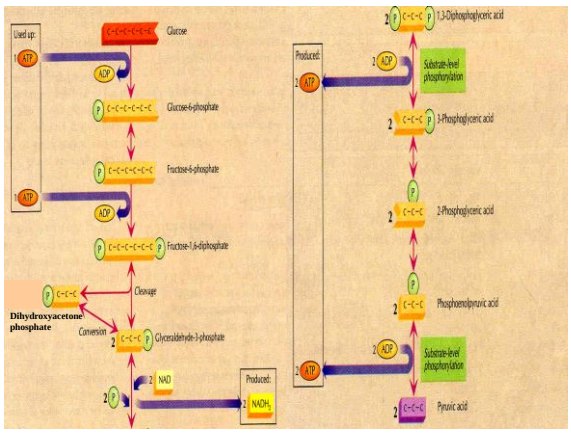
Monosaccharides, amino acids, glycerol and fatty acids are then converted to other compounds that can enter the main dissimilatory pathways of a cell, such as glycolysis, citric acid cycle, etc.

Overall general scheme showing some of the dissimilatory pathways used by organisms for the breakdown of complex nutrients.



Glycolysis

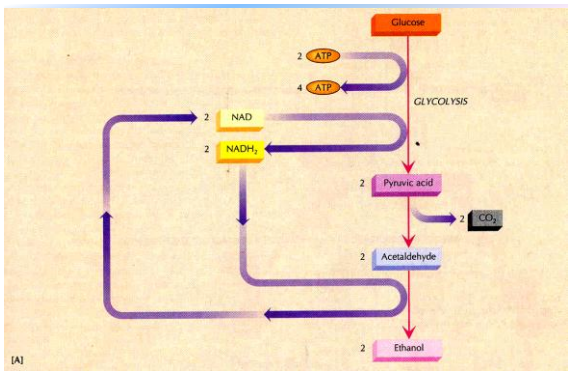
A molecule of glucose is broken down to two molecules of pyruvic acid. Two ATP molecules are used up in the process; however, four ATP molecules are produced by substrate-level phosphorylation. Thus there is a net gain of two ATP molecules. Two molecules of $NADH_2$ are also produced, and these must be oxidized back to NAD so that glycolysis can continue to break down more glucose molecules.



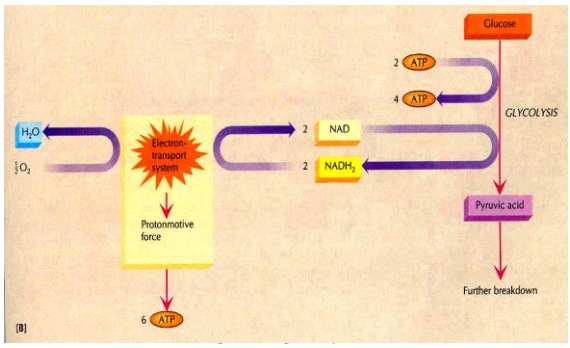
Regeneration of NAD

- Living organisms use one of two methods to regenerate NAD from $NADH_2$ – namely, *fermentation* and *respiration.*
- Fermentation is an oxygen-independent process in which the $NADH_2$ that is produced during glycolysis or another dissimilatory pathway is used to reduce an organic electron acceptor made by the cell itself.
- Fermentation is very inefficient process for extracting energy, because the end products still contain a great deal of chemical energy.
- An example is the ethanol produced by the yeasts – proof of its high energy content is the fact that ethanol is an excellent fuel and liberates a great deal of heat when burned.

Methods used by yeast cells to regenerate NAD from the $NADH_2$ produced in glycolysis. [A] In alcoholic fermentation the $NADH_2$ reduces acetaldehyde to ethanol.

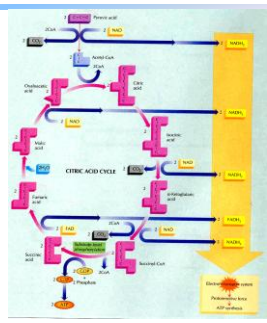


[B] In aerobic respiration $NADH_2$ serves as the electron donor for an electron transport system, which in turn generates a protonmotive force that drives the synthesis of ATP.



Citric Acid Cycle

Initially, pyruvic acid from glycolysis is oxidized to acetyl-CoA, which then undergoes a condensation with oxaloacetic acid to form citric acid. This condensation is the first reaction in a cyclic series of reactions that regenerates oxaloacetic acid. $NADH_2$ and $FADH_2$ molecules are produced at various steps and can serve as electron donors for an electron-transport system that generates a protonmotive force. GTP is generated by substrate level phosphorylation; energetically, it is equivalent to ATP.



Regeneration of NAD

- Another process, called *respiration,* is much more efficient than fermentation for yielding energy.
- Respiration is the process of regenerating NAD by using $NADH_2$ as the electron donor of an electron transport system.
- If oxygen is the terminal electron acceptor of the electron for the electron-transfer system, the process is called *aerobic respiration.*
- However, many bacteria can carry out respiration under anaerobic conditions by using a terminal electron acceptor other than oxygen, such as nitrate or sulfate. This process is termed *anaerobic respiration.*
- Respiration has a great advantage over fermentation: *not only is NAD regenerated, but the electron-transport system generates a protonmotive force that can be used to drive synthesis of additional ATP molecules.*

Regeneration of NAD

Citric Acid Cycle

The dissimilation of glucose by aerobic organisms does not normally stop with the production of pyruvic acid. Further breakdown begins with the oxidation of the pyruvic acid by NAD to *acetyl-CoA* (a two carbon acid, acetic acid, linked to coenzyme A). Each of the two resulting molecules of $NADH_2$ can serve as third electron donor for a electron-transport system , with consequent ATP synthesis

Regeneration of NAD

- Citric Acid Cycle
  - In the case of yeast cells respiring aerobically with glucose, the net yield of ATP from complete breakdown of one glucose molecule is 38 ATP molecules.
  - Thirty four of these are formed when $NADH_2$ and $FADH_2$ serve as electron donors for the yeast's cells electron-transport system.
  - The remainder are formed via substrate level phosphorylation during glycolysis and the citric acid cycle.
  - In sharp contrast to aerobic respiration is the yield of ATP from fermentation when yeast cells are grown anaerobically, where the yield is only two ATPs per molecule of glucose.
  - From this you can see that aerobic respiration is far more efficient than fermentation in extracting the chemical energy of glucose.

Microbial Metabolism

Energy – Requiring
Biochemical Processes

Overview

- Just as an electric generator provides power to machinery, the exergonic reactions provide energy for cellular activities.
- These activities, or biochemical processes, are endergonic (energy-requiring)
- The energy they use is supplied by adenosine triphosphate (ATP), or by some other energy source such as guanosine triphosphate (GTP), uridine triphosphate (UTP), or a protonmotive force.
- Organisms use this energy to fuel the many endergonic reactions required for the life of the cell.
- For instance, ATP is needed for biosynthesis of the various chemical components of the cell – deoxyribonucleic acid (DNA), ribonucleic acid (RNA). enzymes, cell-wall peptitoglycan, and the phospholopids of the cell membrane.

Overview

- ATP can energize amino acids, nucleotides, monosaccharides, and fatty acid precursors, which then enter their respective pathways as building blocks of proteins, carbohydrates, and lipids.
- Besides biosynthesis, a cell also needs ATP or other forms of energy for processes such as motility and the active transport of nutrients across the cell membrane.

Energy Utilization for Biosynthetic Processes

- The cell is a marvelous chemical engineer, busily engaged in assembling the intricate molecules of life.
- Indeed, many of the chemical substances made easily by living cells are so complex that they cannot yet be artificially synthesized by chemists in the laboratory.
- Microorganisms show great diversity in their nutritional requirements.
- These differences are a reflection of their varying biosynthetic abilities.
- For instance, some microorganisms can synthesize all their cellular constituents from simple inorganic compounds.
- Others with less biosynthetic ability must be provided with sugars, amino acids, and vitamins.

Energy Utilization for Biosynthetic Processes

- An example of a microorganism that has relatively simple nutritional requirements is the bacterium *Escherichia Coli.* This bacterium can grow in a medium containing only glucose and a few inorganic compounds, including a source of nitrogen such as ammonium sulfate $[(NH_4)_2SO_4]$
- How does *E. coli* make all these substances?

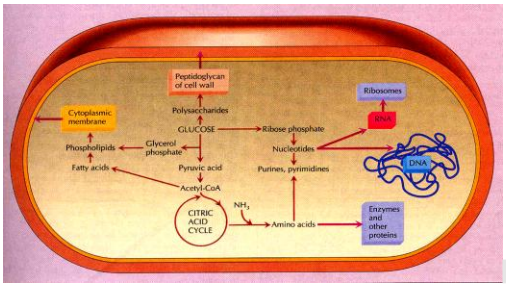
- Some generalizations can be made about the various pathways, because all of them share fundamental features:

1. A biosynthetic pathway begins with the synthesis of biochemical building blocks needed to make more complex substances.

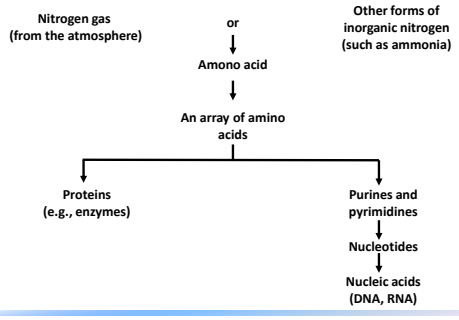
2. The building blocks are then energized, usually with the energy of ATP molecules. This energy is needed to establish the covalent bonds that subsequently will link the building blocks.

3. The energized building blocks are linked together to form complex substances that become structural or functional parts of the cell.

Schematic illustration of how the bacterium *Escherichia coli,* when grown in a medium containing glucose plus ammonium sulfate and other inorganic salts, can synthesize the biochemical building blocks for construction of proteins, polysaccharides, lipids, and nucleic acids, as well as all cell structures. Some structural and functional parts of the cell are indicated by the color boxes



Biosynthesis of Nitrogenous Compounds

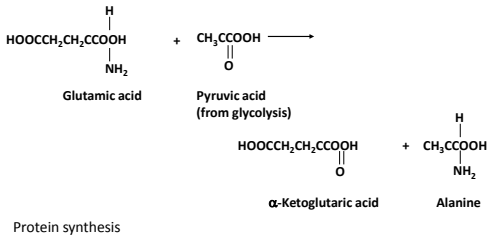


Biosynthesis of Nitrogenous Compounds

- $N_2 + 6e^- + 6H^+ + 16ATP \rightarrow 2NH_3 + 16ADP + 16$ phosphate

Biosynthesis of Amino Acids and Proteins

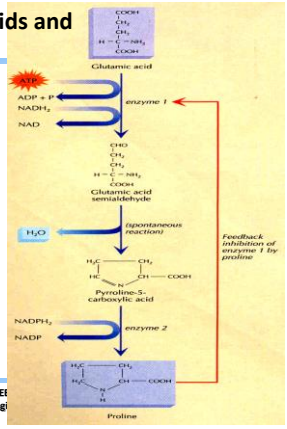
α-Ketoglutaric acid + $NADPH_2$ + NH_3 → glutanic acid + NADP + H_2O



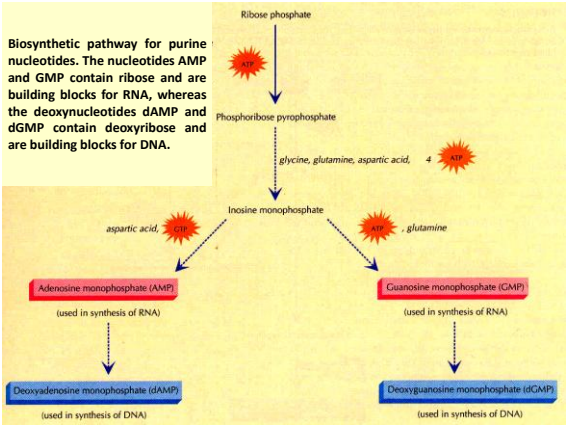
Protein synthesis

Biosynthesis of Amino Acids and Proteins

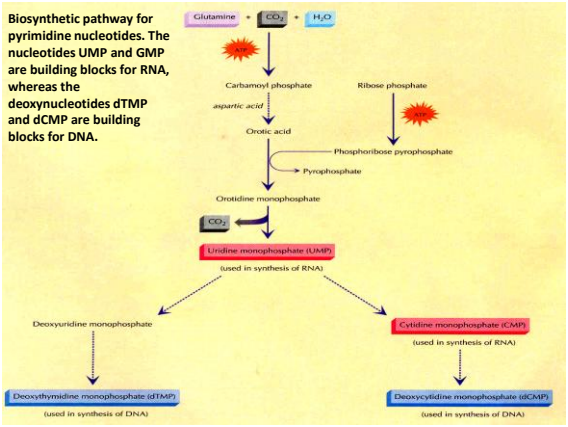
The biosynthesis of the amino acid proline from glutamic acid in *E. Coli*. Note the utilization of metabolic energy in the form of ATP. Over production of proline is prevented by feedback inhibition, in which increasing levels of proline inhibit the activity of enzyme 1.



Biosynthetic pathway for purine nucleotides. The nucleotides AMP and GMP contain ribose and are building blocks for RNA, whereas the deoxynucleotides dAMP and dGMP contain deoxyribose and are building blocks for DNA.



Biosynthetic pathway for pyrimidine nucleotides. The nucleotides UMP and GMP are building blocks for RNA, whereas the deoxynucleotides dTMP and dCMP are building blocks for DNA.



Biosynthesis of Carbohydrates

Carbon dioxide
(from the atmosphere)
↓
Triose
(3-carbon monosaccharode)
↓
Pentoses and Hexoses
(5-and 6-carbon monosaccharides)

- Nucleotides (which contain ribose and deoxyribose) → RNA and DNA
- Polysaccharides (such as the peptidoglycan of the bacterial cell wall, cellulose, starch, and numerous other compounds)

Biosynthesis of Carbohydrates

CO_2 + Ribulose biphosphate ⟶ 2 phosphoglyceric acid

1 Carbon atom; 5 Carbon atoms; 3 Carbon atoms each

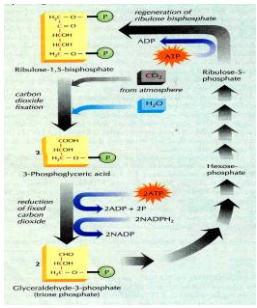
$6CO_2 + 12NADH_2 + 18ATP + 12H_2O \longrightarrow C_6H_{12}O_6 + 12NAD + 18ADP + 18$ phosphate

Glucose + ATP + 2NADP ⟶ Ribose-5-phosphate + CO_2 + ADP + $2NADPH_2$

Glucose + ATP + UTP ⟶ UDP-glucose + ADP + pyrophospate

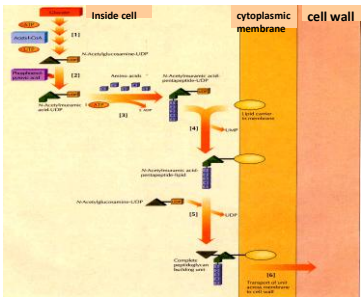
Ribose phosphate + ATP ⟶ Phosphoribose pyrophosphate + AMP

The Calvin cycle for carbon dioxide fixation in autotrophic organisms



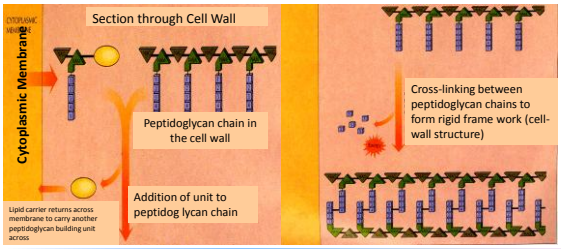
Biosynthesis of a peptidoglycan building unit

Note the expenditure of energy in form of the high energy transfer compounds ATP, acetyl-CoA, UTP, and phosphoenolpyruvic acid. The lipid carrier enables the unit to pass from the inside of the cell across the cytoplasmic membrane to the cell wall.

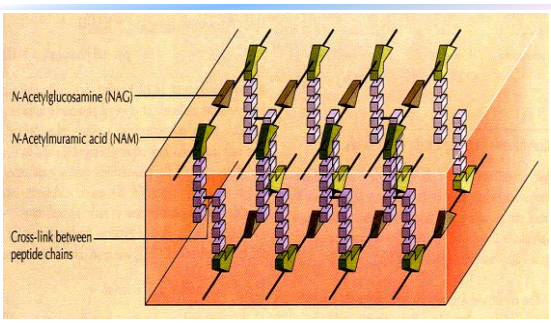


Biosynthesis of a peptidoglycan building unit

Having crossed the cytoplasmic membrane, the peptidoglycan unit is added to an existing peptidoglycan strand. Eventually the various peptidoglycan strands are cross-linked. The chemical bond between amino acids 4 and 5 on each pentapeptide chain is broken by the enzyme transpeptidase, and the energy that is liberated is used to establish cross-links as shown.



Three-dimensional arrangement of the completed peptidoglycan structure of the bacterial cell wall. Note that not all the tetrapeptides are cross-linked.



Biosynthesis of Lipids

Glucose
↓ Glycolysis
Pyruvic acid
↓
Acetyl-coenzyme A and malonyl-coenzyme A
↓
Long-chain fatty acids
↓ ← Glycerol phosphate
Phospholipids

Biosynthesis of Lipids

Acetic acid + ATP + Coenzyme A → Acetyl-CoA + AMP + pyrophosphate

Acetyl-CoA + CO_2 —ATP→ Malonyl-CoA

(2 carbon atoms) (1 carbon atom) (3 carbon atoms)

Biosynthesis of Long-Chain fatty acids

Acetyl-protein + Malonyl-protein → Butyryl-protein + CO_2

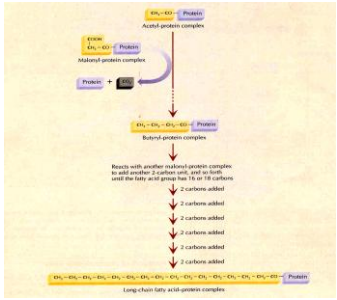
(2 carbon atoms) (3 carbon atoms) (4 carbon atoms) (1 carbon atom)

Dihydroxyacetone phosphate + $NADH_2$ → glycerol phosphate + NAD

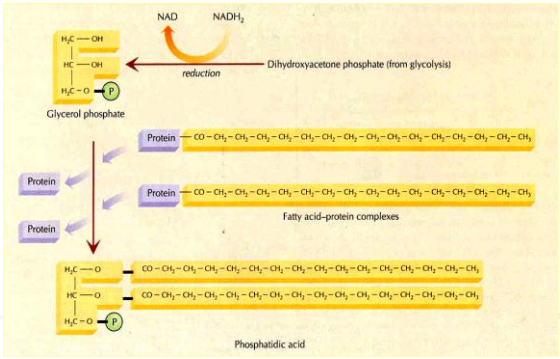
Phosphatidic acid + CTP + serine → phosphatidylserine + pyrophosphate + CMP

Biosynthesis of Long-Chain fatty acids

The biosynthesis of fatty acids proceeds by the sequential addition of two-carbon units a long-chain fatty acid group is formed, usually having 16 or 18 carbon atoms.



Biosynthesis of phosphatidic acid, a simple phospholipid

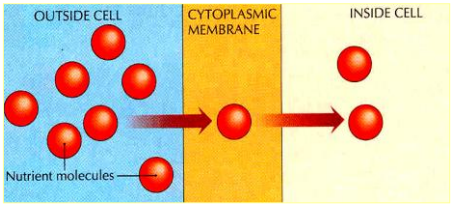


Energy Utilization for Processes other than Biosynthesis

- Transport of nutrients into cells
  - Facilitated diffusion
  - Active diffusion
- Motility

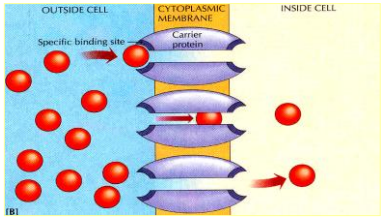
Simple Diffusion

In simple diffusion nutrient molecules pass freely across a cell membrane



Facilitated Diffusion

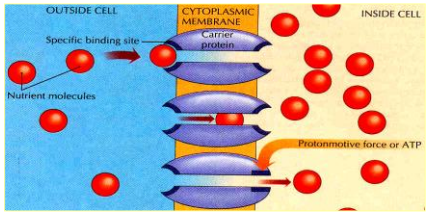
In facilitated diffusion they bind to a specific site on a carrier protein and are transported across the membrane. In either case, when the concentration inside the cell becomes equal to that outside, molecules will leave the cell at the same rate they enter the cell.



Active Transport

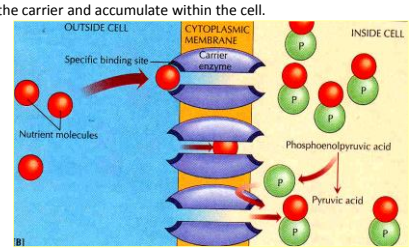
Active transport of nutrient molecules across the cell membrane results in a higher concentration inside the cell than outside.

In one type of active transport, the energy of ATP or the protonmotive force distorts the binding site of the carrier, making it difficult for a molecule to leave the cell once it has entered.



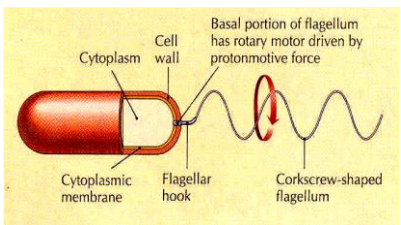
Active Transport

In a second type of active transport, the carrier is an enzyme that adds a phosphate group from phosphenolpyruvic acid to the nutrient molecules during transport. The altered nutrient molecules no longer fit the binding site on the carrier and accumulate within the cell.



Motility

The rotary motor that drives a bacterial flagellum is associated with the disks of the basal portion of the flagellum and is powered by the protonmotive force.



Cycling Ideas

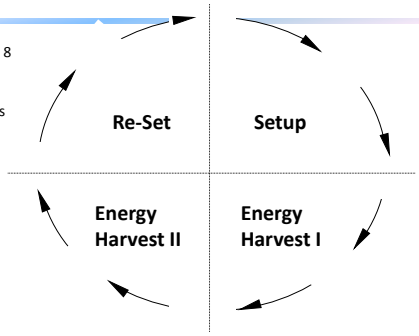
- cycles turn but only can keep turning with outside input = acetyl CoA
  - a water wheel turns because water is added
    - stopping the flow of water stops the wheel
- cycles have an output = energy compounds
  - a water wheel is attached to a shaft and can produce work (grind wheat, etc)
- cycles that are broken do not work!
  - A missing component seizes up the wheel

Learning the CAC

- Know the ideas about cycles
- Look for patterns and memory devices
- Think of a cycle as a game
  - a game has to be set up
  - a game is played
  - if another round is desired, the game is re-set

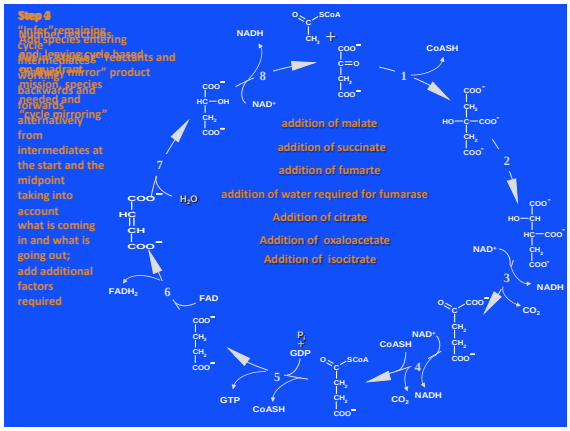
Learning the CAC

Step 1
Draw a circle with 8 reactions divided into 4 **quadrants.**
Label quadrants as per their role.

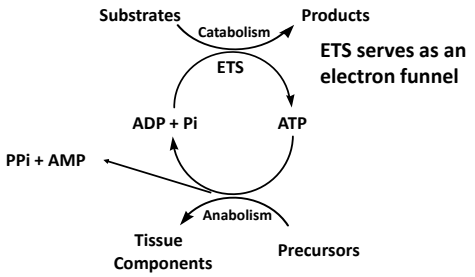


Quadrant Missions

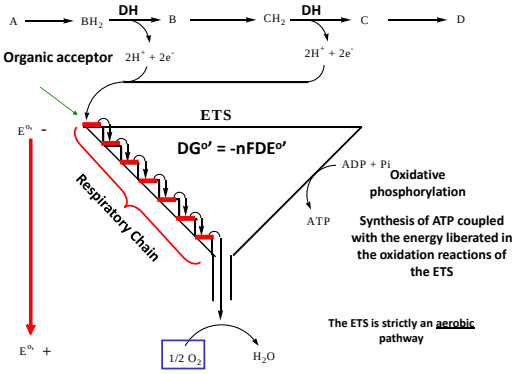
- Setup = combination of feed-in substance (acetyl CoA) and cycle substance (oxaloacetate); release of CoASH for re-cycling; preparation of metabolite for energy extraction
- Energy Harvest I = oxidative decarboxylation = loss of CO_2; production of NADH
- Energy Harvest II = release of CoASH for re-cycling; production of GTP and $FADH_2$
- Re-Set = return to oxaloacetate; 'mirror' energy harvest



Metabolism: An Overview

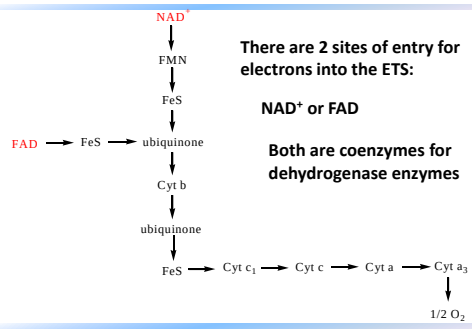


A catabolic pathway (oxidative) **What do dehydrogen ases do?**



Reserved Slides

Electron Carriers

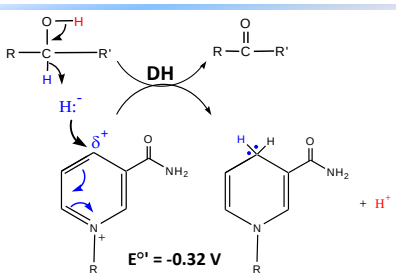


There are 2 sites of entry for electrons into the ETS:

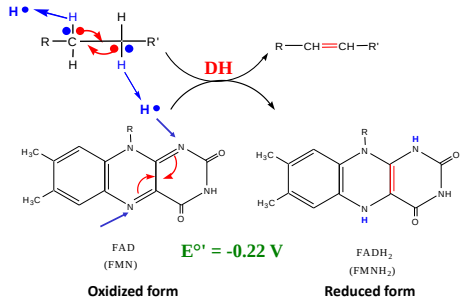
NAD^+ or FAD

Both are coenzymes for dehydrogenase enzymes

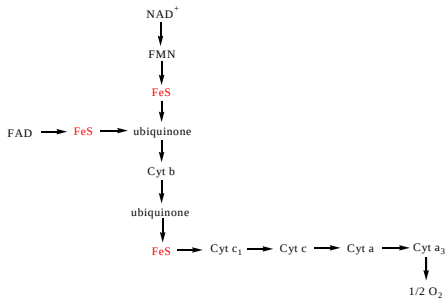
NAD^+



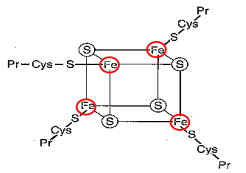
FAD (FMN)



Nonheme Iron (FeS)

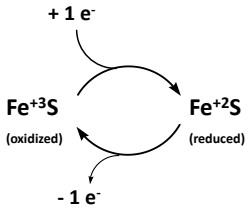


Nonheme Iron (Fe-S Centers)



Fe_4S_4 center. In Fe-S centers, the Fe is chelated to free sulfur (S) atoms, and to cysteine sulfhydryl groups on proteins. Other Fe-S centers contain Fe_2S_2. The protein subunits are sometimes called non-heme iron proteins. When these proteins are treated with acid, the free sulfur produces hydrogen sulfide (H_2S)—the familiar smell of rotten eggs.

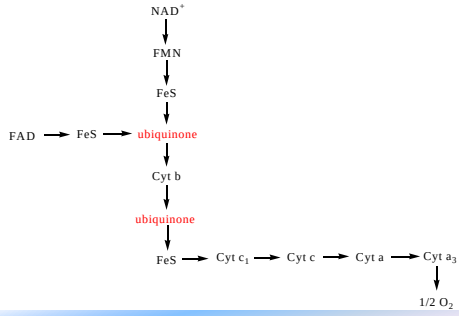
Role of Iron in FeS



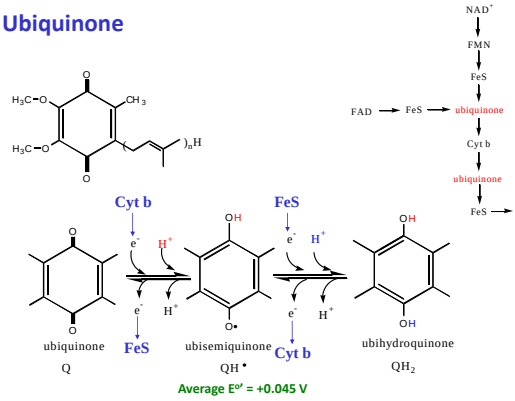
Accept electrons only

Reduction potentials vary

Ubiquinone

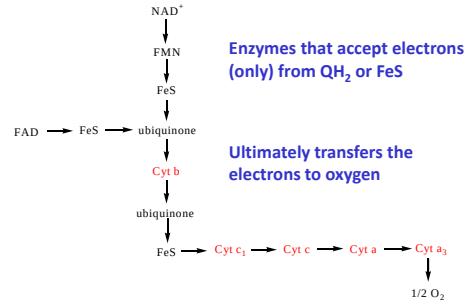


Ubiquinone



Average E°′ = +0.045 V

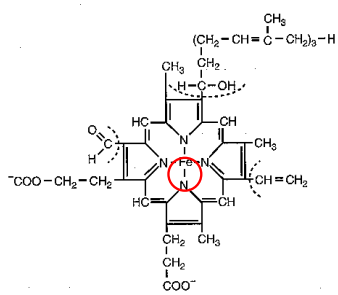
Cytochromes



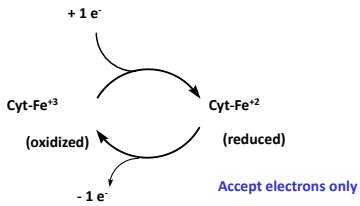
Enzymes that accept electrons (only) from QH_2 or FeS

Ultimately transfers the electrons to oxygen

The Heme in "A" Cytochromes

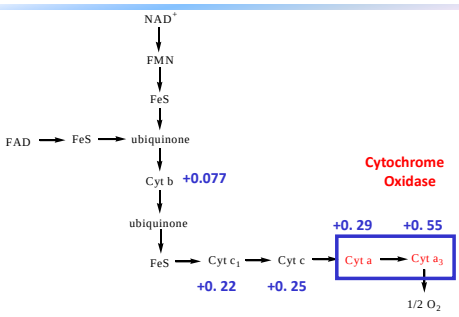


Role of Heme Iron in Cytochromes

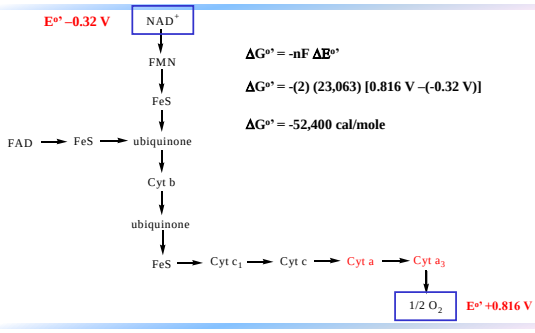


Accept electrons only

Order and Reduction Potentials



Exergonic Nature of the ETS



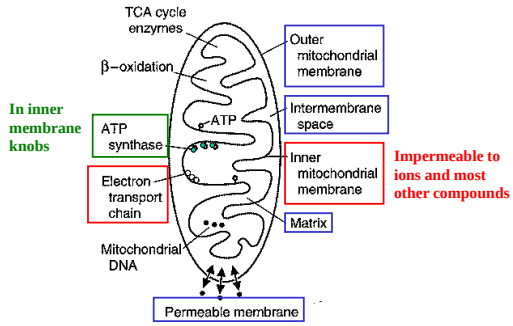
Number of ATP Generated by the ETS

Theoretical:

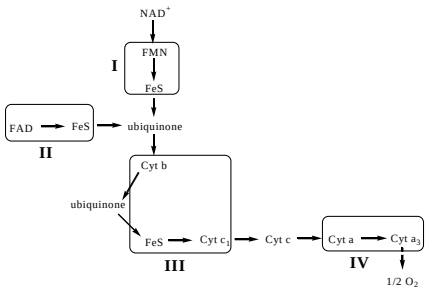
$$\frac{52{,}400}{8{,}000} = 6.5 \text{ per NADH oxidized}$$

Actual: 3 ATP per NADH

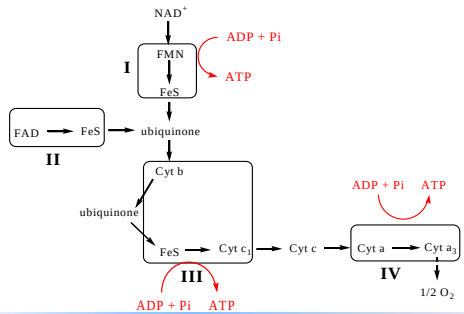
Intracellular Location of the ETS



Mitochondrial Complexes



Sites of ATP Coupling



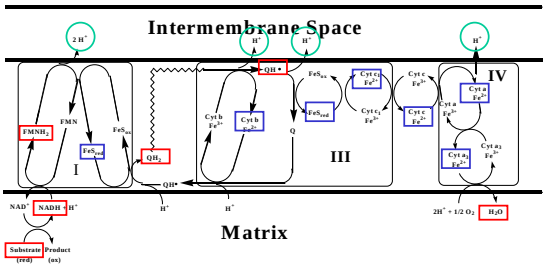
D. Oxidative Phosphorylation

1. P/O Ratios and Sites of ATP Coupling
2. Chemiosmotic Hypothesis

Main Thesis:

The flow of electrons through the ETS translocates protons from the matrix across the inner membrane to the intermembrane space.

Chemiosmotic Hypothesis



Requirements for a Complex to Translocate Protons

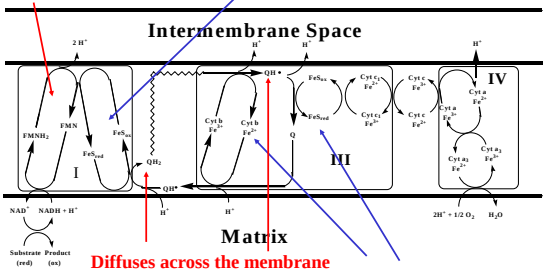
Must have the following alternating pattern of carriers:

1) First must carry <u>both e⁻s and Hs</u> and either:
   a. <u>Span</u> the membrane
   b. <u>Diffuse across</u> the membrane
2) Second must be able to accept e⁻s <u>ONLY</u>

Chemiosmotic Hypothesis

Spans the membrane
Carries both Hs and e⁻s

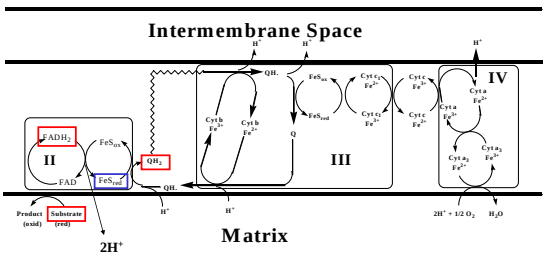
Carries only e⁻s



Diffuses across the membrane
Carries both Hs and e⁻s

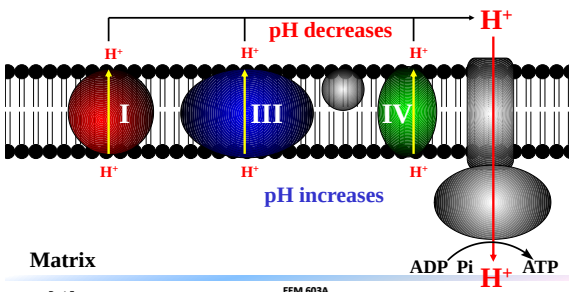
Carry only e⁻s

Chemiosmotic Hypothesis



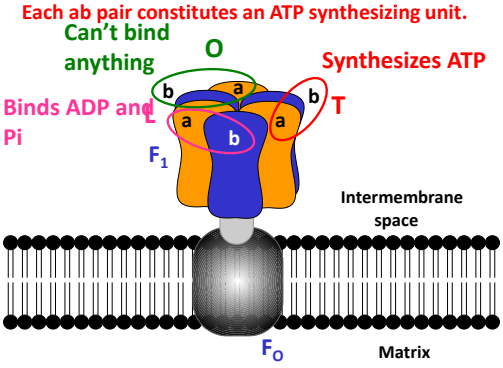
Electrochemical Gradient

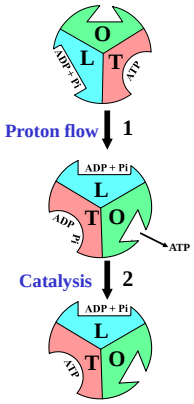
Intermembrane Space



Structure of ATP Synthase

Each ab pair constitutes an ATP synthesizing unit.





Conformational changes:

O ⟶ L
Can now bind ADP and Pi

T ⟶ O
Releases ATP

L ⟶ T
Combines ADP and Pi forming ATP

Respiratory Control: Tight coupling between the rate of electron flow through the ETS and the rate of ATP formation.

ATP formation is the rate limiting process

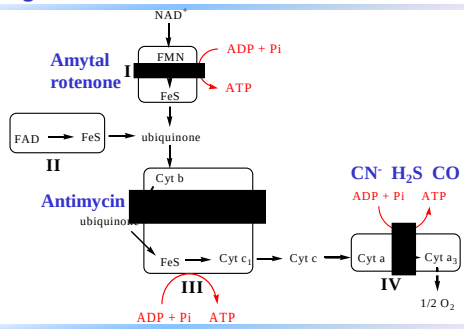
High [ADP]-- Accellerates electron flow and therefore ATP synthesis

Low [ADP]-- Decreases the rate of electron flow

E. Regulation of the ETS

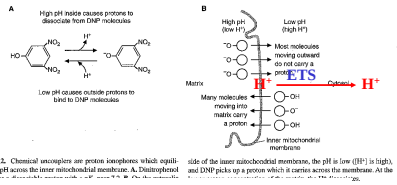
F. Drugs that Affect the ETS

Drugs that Affect the ETS



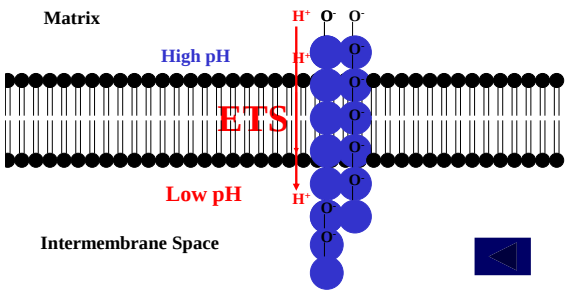
Uncouplers of Oxidative Phosphorylation

Compounds that block ATP synthesis but allow electron flow to continue.



The rate of electron flow would increase.
(increase oxygen consumption and breathing rate)
All the energy would be released as heat.

Mechanism for Uncoupling



End of Part 5